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Potential Technological Use of Reserves of *Jatropha curcas* and *J. macrocarpa* Griseb. Seeds

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Abstract

J. curcas and *J. macrocarpa* are useful for restoring degraded areas and their seeds contain oils for biodiesel production. The aim of the work was to determine the reserve substances in the endosperm and the embryo of *J. curcas* and *J. macrocarpa* which is important in understanding the germination process, the establishment of these species and its industrial employment. Seeds were imbibed in distilled water for 24 h, to facilitate removal of seed coat with the aim to separate the embryo and nutritive tissues. In both species, the endosperm contained aleurone grains consisting of a crystalloid and globoid, lipids of red color and the starch was not observed. Four major fatty acids were determined in *J. curcas* seed: oleic, palmitic, stearic, palmitoleic and oleic fatty acid represents about 70% oil content. Oleic acid was the most abundant in *J. macrocarpa* seeds, while, there was not palmitoleic acid. Seed with predominantly unsaturated fatty acids is ideal for biodiesel industry. The means of the sugar content were: 14.3 μg/mg in endosperm and 104.76 μg/mg in embryo of *J. curcas* and 6.48 μg/mg in endosperm and 59.20 μg/mg in embryo of *J. macrocarpa.* The means of the protein content were: 4.2 μg/mg in endosperm and 45.02 μg/mg in embryo of *J. curcas* and 3.26 μg/mg in endosperm and 31.08 μg/mg in embryo of *J. macrocarpa.* Sugar and protein contents of *Jatropha* seeds were significantly higher in embryo in both species

($p < 0.1$), which suggests early mobilization towards the embryo during imbibition period.

Keywords

Fatty Acids, *J. curcas*, *J. macrocarpa*, Proteins, Seeds, Sugars

1. Introduction

It is widely accepted that *Jatropha curcas* L. and *Jatropha macrocarpa* Griseb. (Euphorbiaceae) are perennial species adapted to marginal conditions not suitable for agriculture and have been exploited for oil and biodiesel production [1]. Both species growing in semi-arid and arid soils and their non-edible seeds have high oil content [2]. The quality of the biodiesel produced depends on the composition of fatty acids. These differ in three characteristics: the size of the hydrocarbon chain, the number of unsaturation and the presence of chemical groups. A good deal of attention has been paid to the fact that biodiesel with a predominance of combined monounsaturated fatty acids, presents high quality [3].

To contribute to the knowledge on seed metabolism in this species, it is essential to know the reserve of macromolecules that are converted into soluble metabolites that are mobilized to be used during growth and respiration. Seeds have been studied as to the chemical composition of their reserves in order to determine their nutritional value and because their reserve constituents have great importance for obtaining industrial products [4].

During the start of germination to the growth of the seedlings, the seedlings should start photosynthetic activity before exhausting its reserves, so as to ensure successful colonization of the environment [5].

Suda and Giorgini [6] investigated a wild poinsettia (*Euphorbia heterophylla* L.) a native plant of tropical and subtropical of America. Lipids, around 60% of seed dry mass, are the major reserve. Proteins comprise about a quarter of seed dry mass. Soluble sugars comprise about 3.6%. Starch was not detected in the endosperm of *E. heterophylla*. Lipid depletion is completed between 72 and 96 hours. Soluble sugars increase in the embryo with no concomitant decrease in the endosperm, suggesting that sugars are mostly originated from the catabolism of lipids.

According to Souza *et al.* [7], *Jatropha* seeds have about 40.33% lipids as well as 20.95% protein and 9.85% starch.

The knowledge of reserve components of the *Jatropha* seed and its disposition in the cells during different periods of germination is useful to understand the process of reserve mobilization and to determine the potentially right time for the extraction of vegetable oil for that species and its subsequent industrial employment. In *J. curcas*, the levels of lipids increased up to 43% at the moment of protrusion. The carbohydrate levels, in both the endosperm and the embryo are minimal during protrusion and shortly after, indicating the maximum use of

carbohydrates in the germination process. Greatest protein synthesis occurs with the greatest moisture gain, with a reduction in and agglomeration of protein bodies during embryo root growth after protrusion. Despite the presence of starch in the endosperm of *J. curcas* starch cannot considered a source of sugars during the period of germination studied [8].

In *J. macrocarpa* no data exist on the biochemical aspects of reserve composition in the seed; furthermore, little information is known about *J. curcas.* The aim of this paper is to determine the reserve substances in the endosperm and the embryo of *J. curcas* and *J. macrocarpa* seeds which is important in understanding the germination process, the establishment of these species and its industrial employment.

2. Material and Methods

Seeds of *J. curcas* and *J. macrocarpa* were collected in a wild population located 30 km south of La Rioja city, Argentina (29.3˚S; 66.8˚W, 438 m above sea level).

Ten *J. macrocarpa* and *J. curcas* seeds were imbibed in distilled water for 24 h to facilitate removal of seed coat to observe the embryo and nutritive tissues.

2.1. Qualitative Identification of Reserve Substances

Anatomical studies were done by longitudinal cuts of endosperms. Ten endosperms seeds of each species were fixed in FAA (50 parts 95% ethanol, 5 parts glacial acetic acid, 10 parts 37% formaldehyde, 35 parts water). After fixation, samples were dehydrated and embedded in Histowax (highly purified paraffin wax blended with polymer additives). A series of longitudinal sections of 12 μm thick were obtained from the sample blocks using a Minot rotary microtome. The sections were triple-stained with hematoxylin, safranin O and fast green FCF, as described by Johansen [9]. The sections were air-dried and mounted in DPX Mountant (Sigma-Aldrich, St Louis, MO, USA). A standard Zeiss Model 16 microscope was used to assess the histological preparations; microphotographs were taken with a Zeiss Axiophot microscope, equipped with AxioCam HRc camera and an image capturing and digitalization system (AxioVision 4.3).

For identify lipid, freehand endosperm sections of *J. curcas* and *J. macrocarpa* were cut from fresh material and treated with Sudan IV; iodine iodine-potassium was used for identification of starch [10]. To determine protein bodies, the sections were immersed in ethyl alcohol to remove oils and contrast the aleurone granules, which were colored with potassium iodine-potassium to identify crystalloids and globoids.

2.2. Quantitative Identification of Reserve Substances in Endosperms and Embryos

J. macrocarpa and *J. curcas* seeds were imbibed in distilled water for 24 h. After that the fresh weight of embryos and endosperms was measured, then macerated in mortar with 0.85% physiological solution. Quantification of reserve substances in seeds were carried by different methods by triplicate experiments.

2.3. Fatty Acids

Total lipids were extracted from a humid biomass of 400 mg of embryos and endosperms, with chloroform/methanol (1:2, v/v) as described by Bligh and [11]. Subsequently, a 0.1 M KCl solution in 50% methanol was added to obtain a lower chloroform phase and an upper aqueous phase. The lower phase, which contains the lipids, was washed twice with KCl solution, dried under nitrogen flow and resuspended in chloroform/methanol (2:1, v/v).

Fatty acids Methyl esters (FAME) were prepared from the total lipid extracts with 10% boron trifluoride (BF_3) in methanol, at 100˚C for two hours and extracted with three volumes of hexane [12]. FAME were separated according to the number of double bonds in silica gel TLC plates impregnated with $AgNO_3$ in acetonitrile (10%, w/v) using hexane/ethyl ether/acetic acid (94:4:2, v/v/v). The FAME bands were located under UV light spraying the plates with a dichlorofluorescein solution. They were analyzed in a Hewlett Packard 5890 gas chromatograph, series II equipped with a methyl silicone column (50 m × 0.2 mm × 0.33 μm), coupled to a flame ionization detector. The temperature of the column was programmed at 180˚C for 25 min and then 3˚C/minute until reaching 250˚C. Detector temperature was 300˚C and injector temperature was 250˚C. The FA peaks were identified using a control mixture provided by Sigma-Aldrich Chemical Co [13].

2.4. Sugars

Sugars were determined by the phenol sulfuric acid method [14]. Glucose was used as a standard at different concentrations (10 μg/ml to 200 μg/ml). Absorbance was measured in spectrophotometer at 495 nm for calibration curve. Following reagents were added: 125 μl Phenol 80% and 625 μl concentrated H_2SO_4 and was incubated 10 minutes at room temperature, then was measured in spectrophotometer at 495 nm. Sugar concentrations (μg/ml) were obtained from standard curve by duplicate experiments.

2.5. Proteins

Proteins were quantified by the method of Bradford [15]. Bovine serum albumin was used as standard. Different dilutions were made (1:10.1:20.1:50). Tubes containing 10 μL of the sample were prepared and 900 μL Bradford reagent was added and absorbance was measured at 595 nm. The protein concentrations were obtained from standard curve by duplicate experiments.

2.6. Statistical Analysis

Analysis of variance (ANOVA) was applied and data were subjected to Multiple Range the Duncan. Test using the software INFOSTAT-UNC.

3. Results

3.1. Qualitative Identification of Reserve Substances

Anatomical cuts showed the endosperm with compactly arranged isodiametric

parenchyma cells of similar size. In both species these cells contained abundant protein bodies. These aleurone grains in *J. curcas* showed a different size in the same cells and in different cells (Figure 1(a)) and generally have a smaller crystalloid than those present in parenchymal cells of *J. macrocarpa* which are fairly uniform in size (Figure 1(b)). Protein bodies in these species show two inclusions: globoids that stain reddish-brown constituted by phytin, protein rich in phosphorus and crystalloids that remain transparent, constituted by other proteins such as albumin (Figure 1(a), Figure 1(b)).

The histochemical tests corroborate the difference between the aleurone grains of both species. The reddish coloration produced by the reaction of the lipids with the Sudan IV allowed detecting a large amount of lipids throughout the cytoplasm of the endosperm cells. Starch was not detected in the endosperm or the embryo of both species (data not shown).

3.2. Quantitative Identification of Reserve Substances

3.2.1. Fatty Acids

Fatty acids composition in the embryo and endosperm of both species was expressed in the corresponding chromatograms (Figure 2, Figure 3). Fatty acids percentages obtained from the chromatograms were presented in Figure 4. Palmitic acid (16:0) was present into the endosperm and into the embryo of both seeds. Small concentrations of palmitoleic acid (16:1Δ9) was only present in the endosperm of *J. curcas*. Octadecanoic acid (stearic acid) (18:0) and oleic acid (18:1) was present into seeds of both species. Oleic acid is the predominant fatty acid in seeds of both species. Within the saturated fatty acids, palmitic acid had greater percentage in the endosperm of *J. curcas* than *J. macrocarpa,* while stearic acid was greater quantity in the endosperm of *J. macrocarpa* than *J. curcas*. Nonadecanoic acid ($19:0_{(cyclopropane)}$) was only found in endosperms and embryos of *J. macrocarpa*, whereas fatty acids (more than 18 C) not identified with the standard system used was absent in embryos of *J. curcas* and present in the endosperm and in the embryo of *J. macrocarpa*.

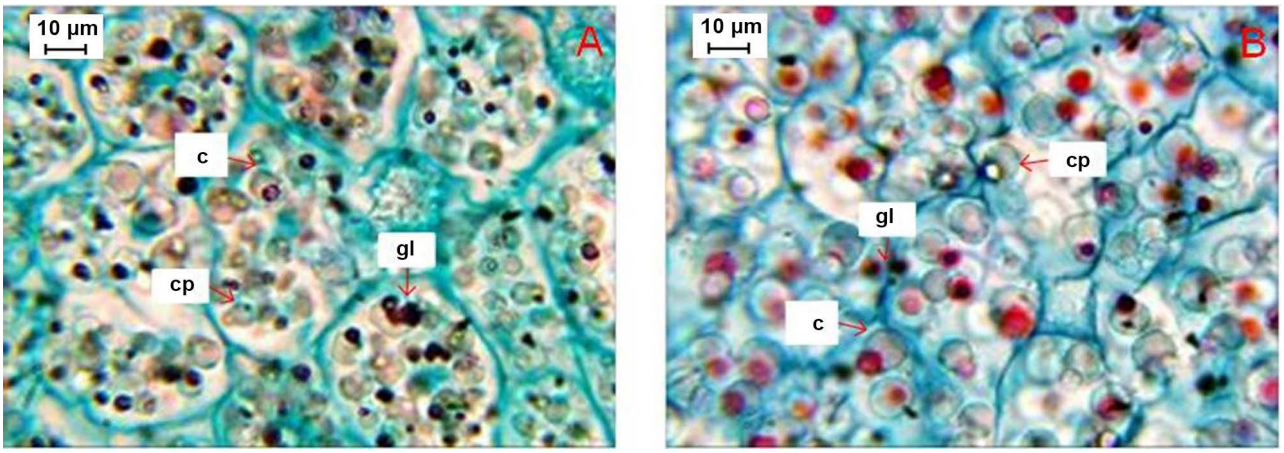

Figure 1. Endosperm cells with lipids and aleurone grains stained with hematoxylin, safranin O and fast green FCF. (a) *J. curcas*; (b) *J. macrocarpa.* Scale bar = 10 µm. Note: ag: aleurone grains; c: crystalloid; gl: lipids.

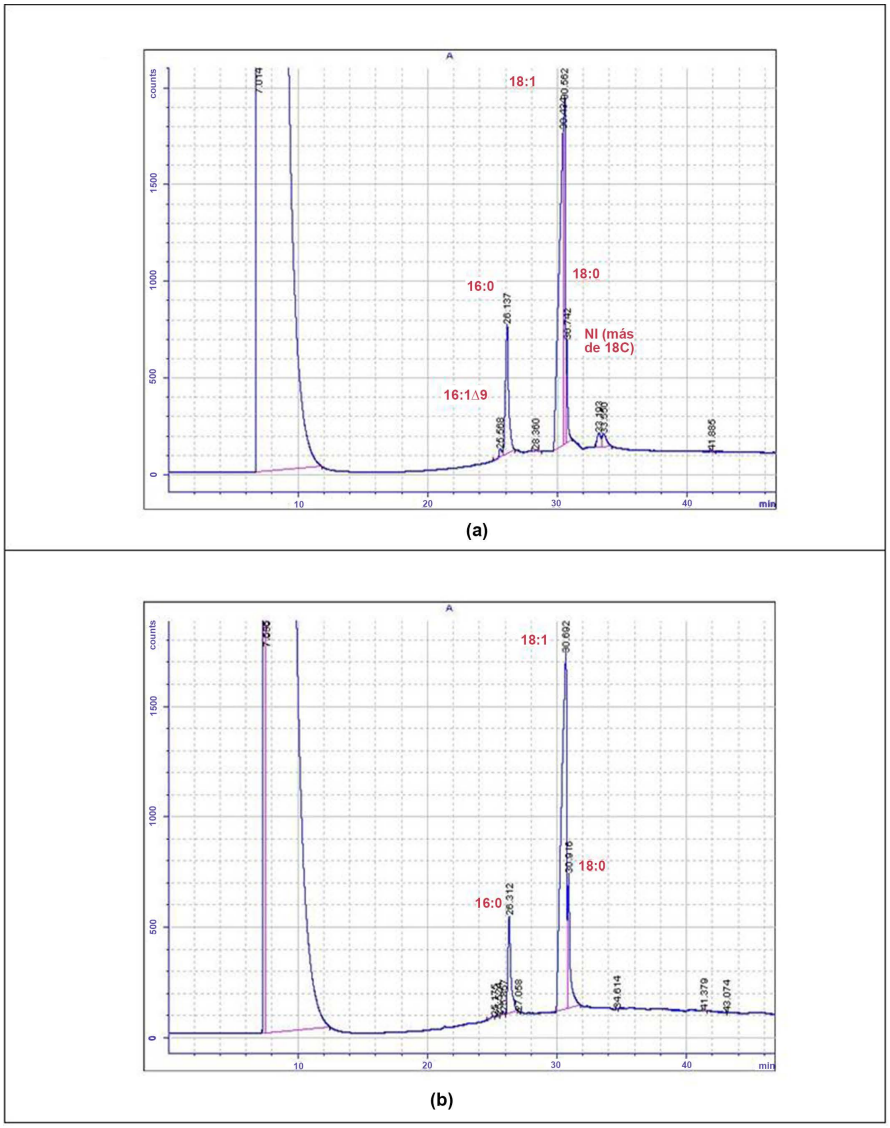

Figure 2. Fatty acid composition (FA) of *Jatropha curcas* seeds. The analysis was performed using a gas chromatograph (GC) coupled to a flame ionization detector. (a) Endosperm FA pattern; (b) Embryo FA pattern. Values are from triplicate experiments. Note: 16:0, palmitic acid; 16:1Δ9, palmitoleic acid; 18:0, stearic acid; 18:1, oleic acid; NI, not identified with the standard system used.

3.2.2. Sugars

Sugar contents of *Jatropha* seeds were significantly higher into embryo in both species ($p < 0.1$) which suggests early mobilization towards the embryo during imbibition period.

Significant differences were shown in favor of *J. curcas* embryo, but no significant differences were observed in the concentration of endosperm sugars ($p < 0.1$) (Table 1).

3.2.3. Proteins

Protein content was statistically higher in the embryo than in the endosperm in both species ($p < 0.1$). In comparison between species, there was significant

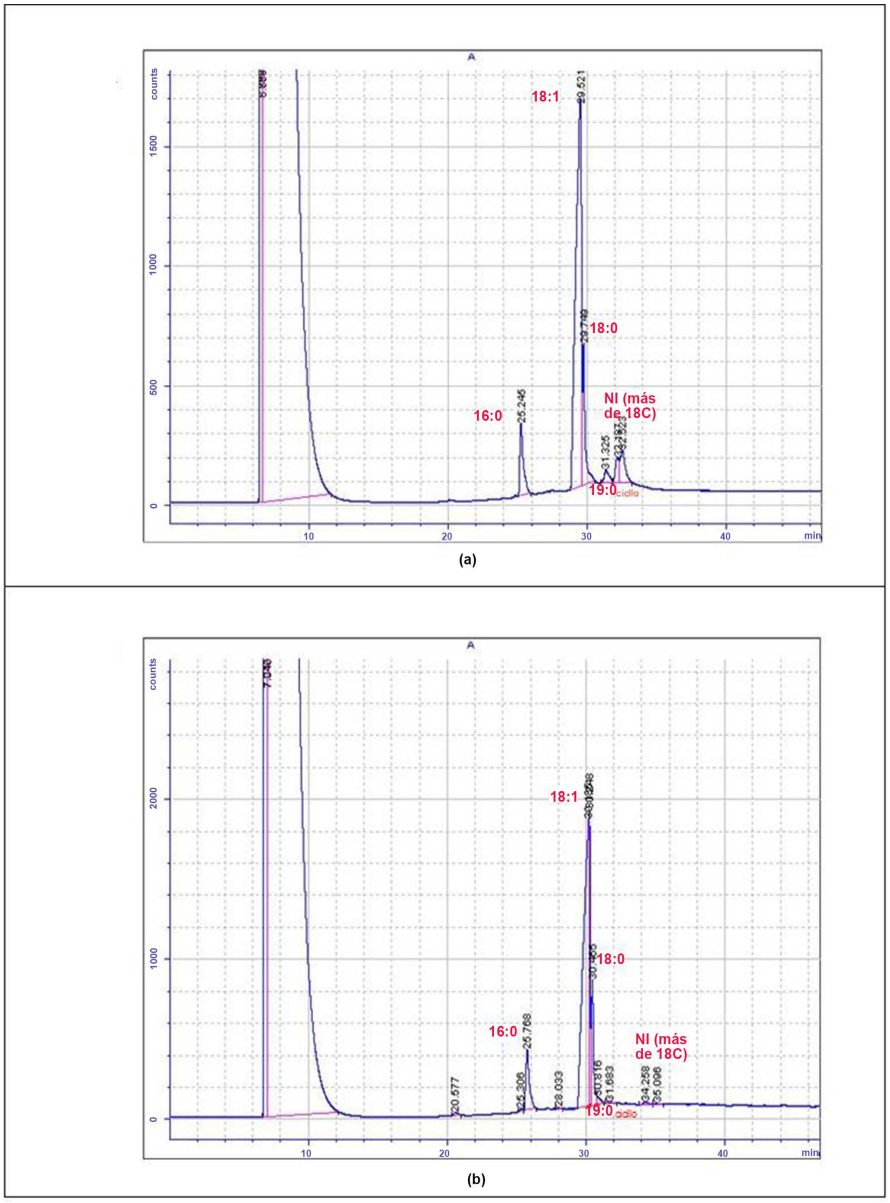

Figure 3. Fatty acid composition (FA) of *Jatropha macrocarpa* seeds. The analysis was performed using a gas chromatograph (GC) coupled to a flame ionization detector. (a) Endosperm FA pattern; (b) Embryo AG pattern. Values are from triplicate experiments. Note: 16:0, palmitic acid; 18:0, stearic acid; 18:1, oleic; $19:0_{(cyclopropane)}$, nonadecanoic acid; NI, not identified with the standard system used.

differences in favor of *J. curcas* in the embryo, however there were no significant differences in the endosperm, similar to sugars ($p < 0.1$) (Table 2).

4. Discussion

The *J. curcas* and *J. macrocarpa* chemical composition seeds can explain biochemical, physiological, cellular and ecology aspects which aids in understanding the different adaptation strategies of the species to their natural environment [16] [17].

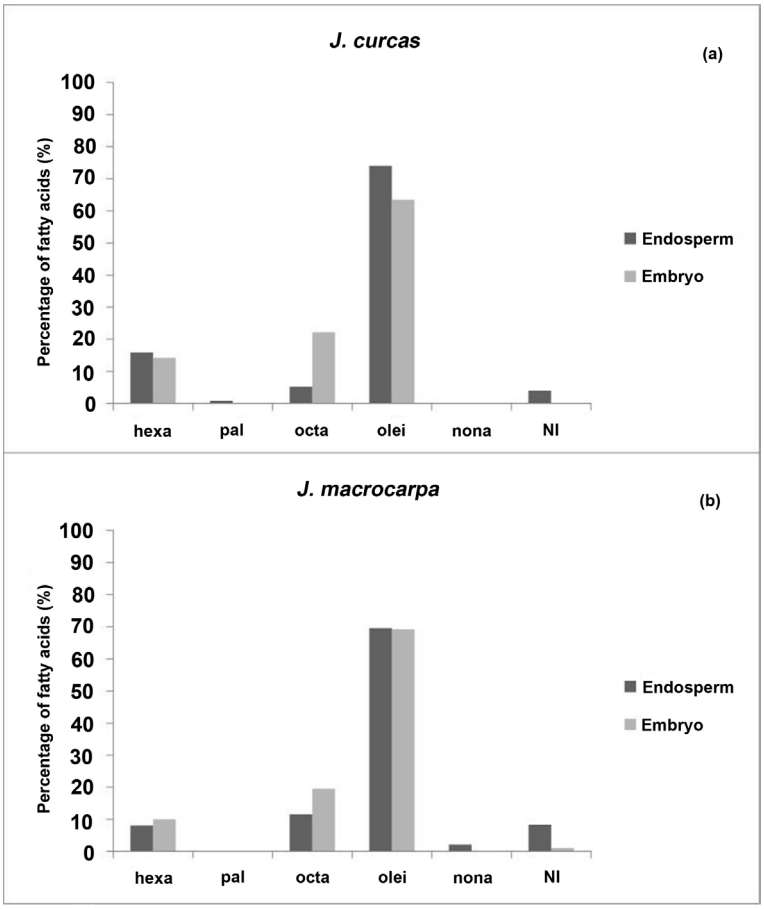

Figure 4. Percentages of fatty acids (FA) in embryo and endosperm of *J. curcas* (A) and *J. macrocarpa* (B). Note: NI, not identified with the standard system used.

Table 1. Content of sugars in endosperm and embryo of *J. curcas* and *J. macrocarpa.* Values are from triplicate experiments. Statistically analyzed between species and between tissues. Different letters used after Means ± standard error are significantly different ($p < 0.1$) as determined by Duncan's multiple range test.

Sugar content	*Jatropha curcas*		*Jatropha macrocarpa*	
	Endosperm	Embryo	Endosperm	Embryo
µg/ml	60.33	40.33	21	20
µg sugar/mg tissue	14.3± 2.43c	104.76± 7.7a	6.48± 3.3c	59.20± 12.9b

Table 2. Content of proteins in endosperm and embryo of *J. curcas* and *J. macrocarpa.* Values are from triplicate experiments. Statistically analyzed between species and between tissues. Different letters used after Means ± standard error are significantly different ($p < 0.1$) as determined by Duncan's multiple range test.

Protein content	*Jatropha curcas*		*Jatropha macrocarpa*	
	Endosperm	Embryo	Endosperm	Embryo
µg/ml	35.5	34.66	21.16	21.16
µg protein/mg tissue	4.2 ± 0.38c	45.02± 3.4a	3.26 ± 0.4c	31.08 ± 6.84b

Starch which serve as reserve carbohydrates in a number of seeds such as *Caesalpinia echinata* and *Caesalpinia ferrea* [18]. In this report was not detected starch in *J. curcas* and *J. macrocarpa* seeds. This finding is consistent with another study in *Euphorbia heterophylla* (Euphorbiaceae) [6]. However, starch reserve accumulated into *J. curcas* seeds from Paraíso, Brasil [7] and from India [19] was very little. Despite the presence of starch in the endosperm of *J. curcas* L. seeds from Fortaleza, Brazil, this concentration remained stable during the germination [8]. Consequently, starch cannot be considered a source of sugars during the germination period in the species under study.

According to other authors, information about morphology, chemical composition and reserve mobilization is important in understanding the establishment of native and exotic species [16] [20]. It is essential the knowledge of chemical composition *Jatropha* seeds because it is important to know the composition of fatty acids in the oil, which affect the quality of biodiesel production [3]. The main metabolic substance of economic interest in the seeds of *Jatropha* are the lipids, not for any nutritional value but because it is a raw material for the bio-fuel industry [4]. From the results of the biochemical studies it was seen that the hexadecanoic, octadecanoid and oleic fatty acids was present in the endosperm and the embryo of *J. curcas* and *J. macrocarpa* seeds. Oleic fatty acid represents about 70% oil content in both species. Small concentrations of palmitoleic acid were only present in the endosperm of *J. curcas* being the first study where this fatty acid was determined in this species. Other authors were detected palmitoleic fatty acid in seeds of *J. macrocarpa* from the North Argentine [21] and in wild plants from La Rioja [1]. Other like, nonadecanoic acid was only found in endosperms and embryos of *J. macrocarpa*. A very interesting fact is that seed of both species with predominantly unsaturated fatty acids is ideal for biodiesel industry, coincident with those reported previously by Pramanik [22] Achten *et al.* [2]. Seed contains high percentage of unsaturated fatty resulting in characteristically low levels of free fatty acids which improves storability [23].

According to Linder [24] saturated fatty acids store more energy per carbon than unsaturated fatty acids; however, unsaturated fatty acids have much lower melting points than saturated fatty acids. Thus, seeds with higher proportions of unsaturated fatty acids should be able to germinate earlier and grow more rapidly at low temperatures. Probably, those mentioned above do not explain the trend observed in *J. curcas.* There are other selection processes acting in this particular case, perhaps the soil humidity is pushing, at least in part, to select unsaturated fatty acids of the seed oil in *J. curcas* [25].

The fatty acids composition may vary due to the genetic load and climate conditions [20]. However, in *J. curcas* and *J. macrocarpa* there is little natural variability for the character "fatty acid composition".

In *Jatropha* seed, nutritional reserves accumulate in the endosperm and very little in the embryo itself [8]. As the fruits of *Jatropha* matured, starch was converted to sugars, resulting in an increase in total sugars the reserve total sugars have to be

converted to soluble sugars [19]. In *J. curcas*, the inner integument has the ability to take up both sucrose and glucose, a rapid uptake of glucose by the developing endosperm fueled glycerolipid synthesis. Thus, the inner integument acts as a transient storage tissue and later the developing endosperm was responsible for synthesis and accumulation of lipids in *Jatropha* seed [26].

In this study, sugar contents of *Jatropha* seeds were significantly higher in embryo in both species, which suggests early mobilization towards the embryo during imbibition period. The sugar reserves are consumed continuously for the production of ATP in the embryo, the consumer center, as that is where there is an accelerated formation of new cells [8]. In both *Jatropha* species contents of endosperm sugar were low in both species, this could explain by gluconeogenesis from lipids and amino acids which were demonstrated in germinating castor bean seed [6]. The ability to successfully complete critical stages in the life cycle such as germination thus enables a rapid crop establishment [27] [28].

The protein reserve is essential to seeds as source nitrogen to embryo development, and for synthesis of new tissues and cells [16] [20]. At the beginning of germination, after imbibition, one of the signs of reactivation of metabolism is the synthesis of proteins with the consequent formation of hydrolytic enzymes that promote the mobilization of reserve substances [8].

We observed that proteins in seeds of *J. curcas* and *J. macrocarpa* were mainly in embryos. Probably, this occurs due protein synthesis in the embryo sustained by amino acids moved from the endosperm to the embryo through synthesis "de novo" during germination. Low content protein in the endosperm probably occurred from the RNAm which is stored at the end of the maturation process [8]. The high concentration of proteins in *J. curcas* compared to *J. macrocarpa* observed in this work is directly related to the reserve of sugars since there is greater availability of hydrolytic that promote the mobilization and degradation of reserve substances. Proteome analysis of the developing endosperm of *J. curcas* allowed to identify different proteins; providing an important glimpse into the enzymatic machinery devoted to the production of C and N sources to sustain embryo development [29].

The concentration of the different reserve components varies among species since they depend on genetics, physiology, and the main regulators that distribute the carbon storage in the seed [20] [30].

5. Conclusion

In conclusion, this study provides evidence that biochemical reserve composition (proteins and sugars) in the seeds could enable a better germination process and crop establishment of *J. curcas*. On the other hand, high unsaturated fatty acids content is ideal for biodiesel industry in both species.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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